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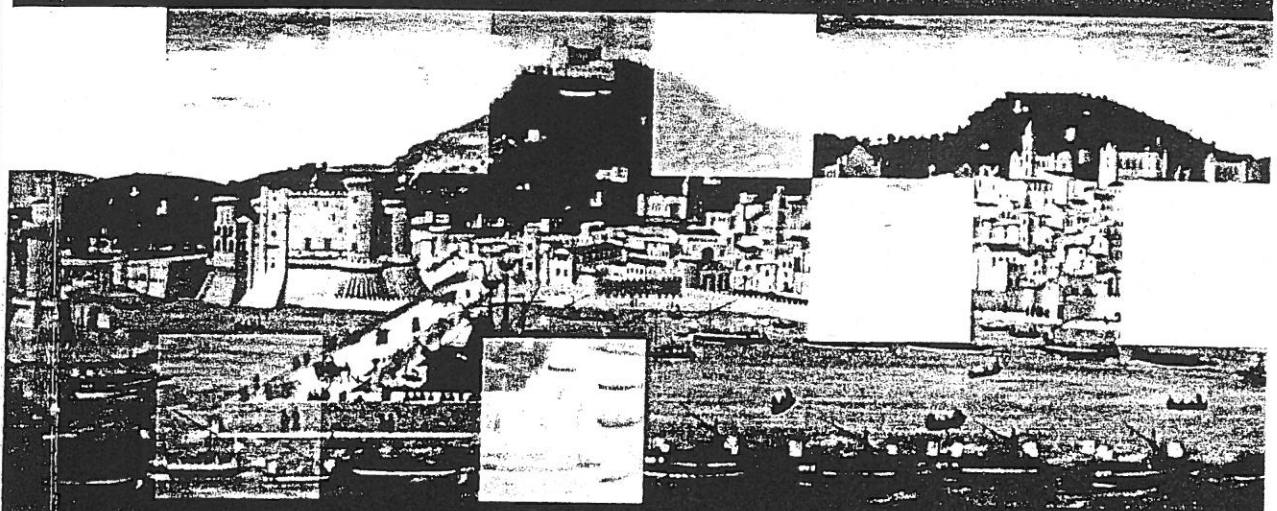

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PROGRAM AND ABSTRACTS

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Expression and binding properties of CBM45 from *Solanum tuberosum*

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Starch binding domains (SBDs) have been found in CBM families 20, 21, 25, 26, 34, 41, 45, 48 and 53 [1], http://www.cazy.org/fam/acc_CBM.html]. Recently a new SBD family, CBM45 was discovered. [2], this family only contains sequences of the plant kingdom. CBM45 occur as an N-terminal tandem domain in only two classes of intracellular enzymes, plastidial amylases (EC 3.2.1.1) and glucan water dikinases (GWDs, EC 2.7.9.4). GWD1 is expressed in photosynthetic organisms and phosphorylates transient and storage starch in the plastids stimulating starch mobilisation. Plastidial α-amylases are presumably involved in the starch turnover, even though the exact role is currently unclear.

In order to probe the domain borders multiple single CBM45 constructs of GWD1 from *Solanum tuberosum* (potato) were made and expressed as a cleavable His-fusion protein in *E. coli*. Protein integrity was tested after removal of the His-tag by differential scanning calorimety (DSC).

Even though CBM45s do not contain cysteines the internal CBM, CBM45-2, is unexpectedly thermostable (T_m of 84°C). Binding properties of the CBM45 were tested by isothermal calorimetry (ITC) and surface plasmon resonance (Biacore) using soluble low molecular weight starch mimetic motifs. The rather low affinity as compared to most other starch binding domains suggests reversible or regulated binding of CBM45-containing enzymes. Similar constructs were fused with YFP to probe the ability of the isolated CBMs *in vivo*. Analyses of tobacco leaves transiently expressing CBM45-YFP-fusions by confocal microscopy suggest in vivo binding of the CBM45 to transient starch granules.

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